

<b>23 RISPERIDONE QUANTITATION AND CONFIRMATION BY LCMS</b>	Page 1 of 4
<b>Division of Forensic Science</b>  <b>TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b>	Amendment Designator:
	Effective Date: 31-March-2004
<p style="text-align: center;"><b>23 RISPERIDONE QUANTITATION AND CONFIRMATION BY LCMS</b></p> <p><b>23.1 Summary</b></p> <p>23.1.1 Risperidone and its proprietary internal standard, R68808, are extracted from biological samples with an acetonitrile precipitation and analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).</p> <p><b>23.2 Specimen Requirements</b></p> <p>23.2.1 One mL blood, fluid or tissue homogenate.</p> <p><b>23.3 Reagents and Standards</b></p> <p>23.3.1 Ammonium acetate</p> <p>23.3.2 Acetic Acid</p> <p>23.3.3 Methanol</p> <p>23.3.4 Acetonitrile</p> <p>23.3.5 Risperidone (Janssen Pharmaceuticals)</p> <p>23.3.6 R68808 (Janssen Pharmaceuticals, internal standard)</p> <p><b>23.4 Solutions, Internal Standard, Calibrators and Controls</b></p> <p>23.4.1 5 mM Ammonium Acetate: Weight 0.19 g ammonium acetate. Transfer to 500 mL volumetric flask and QS to volume with dH<sub>2</sub>O</p> <p>23.4.2 Working standard solution for risperidone (0.01 mg/mL)</p> <p>23.4.2.1 Pipet 100 µl of 1 mg/mL stock solution of risperidone into a 10 mL volumetric flask and QS to volume with acetonitrile</p> <p>23.4.3 Quality Control (QC) standard solution of risperidone (0.01 mg/mL)</p> <p>23.4.3.1 Pipet 100 µl of separate 1 mg/mL stock solution of risperidone (different preparation than calibrators) into a 10 mL volumetric flask and QS to volume with acetonitrile</p> <p>23.4.4 Internal standard working solution</p> <p>23.4.4.1 0.01 mg/mL R68808: Pipet 100 µL of 1 mg/mL R68808 stock solution into 10 mL volumetric flask and QS to volume with acetonitrile</p> <p>23.4.5 To prepare the calibration curve, pipet the following volumes of the 0.01 mg/mL risperidone working solution into appropriately labeled 16 x 125 mm screw cap test tubes. Add 1 mL blank blood to obtain the final concentrations listed below.</p>	

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<table><tr><td>Amount of Standard (µL)</td><td>Final concentration of risperidone (mg/L)</td></tr><tr><td>100</td><td>1</td></tr><tr><td>50</td><td>0.5</td></tr><tr><td>20</td><td>0.2</td></tr><tr><td>10</td><td>0.1</td></tr><tr><td>5</td><td>0.05</td></tr></table>			Amount of Standard (µL)	Final concentration of risperidone (mg/L)	100	1	50	0.5	20	0.2	10	0.1	5	0.05
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100	1													
50	0.5													
20	0.2													
10	0.1													
5	0.05													
23.4.6 Controls														
23.4.6.1 Risperidone Control														
23.4.6.1.1 Pipet 50 µL of the 0.01 mg/mL risperidone QC solution into an appropriately labeled tube. Add 1 mL blank blood to achieve final concentration of 0.5 mg/L.														
23.4.6.2 Negative control. Blood bank blood or equivalent determined not to contain risperidone.														
23.5 Apparatus														
23.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps														
23.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom														
23.5.3 Centrifuge capable of 2000-3000 rpm														
23.5.4 Nitrogen evaporator with heating block														
23.5.5 Vortex mixer														
23.5.6 GC autosampler vials with inserts														
23.5.7 LC/MS: Agilent Model 1100 LC-MSD														
23.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.														
23.5.7.1.1 Elution conditions:														
23.5.7.1.1.1 Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 µM particle size														
23.5.7.1.1.2 Column thermostat: 35° C														
23.5.7.1.1.3 Solvent A: 5 mM ammonium acetate (500 mL) containing 150 µL acetic acid														
23.5.7.1.1.4 Solvent B: acetonitrile														
23.5.7.1.1.5 Isocratic elution, stop time: 4.00 min														
<table><tr><td>Time</td><td>Solv. B</td><td>Flow</td></tr><tr><td>0.00</td><td>80</td><td>0.6</td></tr></table>			Time	Solv. B	Flow	0.00	80	0.6						
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#### 23.5.7.1.2 Spray Chamber

- 23.5.7.1.2.1 Ionization Mode: Electrospray
- 23.5.7.1.2.2 Gas Temperature: 300° C
- 23.5.7.1.2.3 Drying Gas (N<sub>2</sub>): 11.8 L/min
- 23.5.7.1.2.4 Nebulizer pressure: 30 psig
- 23.5.7.1.2.5 Vcap (Positive): 2500 V

#### 23.5.7.1.3 Selected Ion Monitoring (quantitation ions)

Time (min)	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0.00	risperidone	191	115	1.0	High	114
		411				114
		<u>412</u>				114
	RS68808	201				114
		<u>421</u>				114

- 23.5.7.1.3.1 Polarity: Positive
- 23.5.7.1.3.2 Injection volume: 4 µL

### 23.6 Procedure

- 23.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.
- 23.6.2 Prepare calibrators and controls.
- 23.6.3 Add 1 mL case specimens to the appropriately labeled tubes.
- 23.6.4 Add 50 µL 0.01 mg/mL R68808 internal standard working solution to each tube.
- 23.6.5 Slowly, add dropwise 2 mL cold (freezer temperature) acetonitrile to each tube while vortexing. Continuous vortexing, not mere mixing, is essential.
- 23.6.6 Vortex an additional 30 seconds.
- 23.6.7 Place tubes in freezer for at least 30 minutes to facilitate separation.
- 23.6.8 Centrifuge at approximately 2500 rpm for 15 minutes.
- 23.6.9 Transfer top acetonitrile layer to clean conical bottom tubes taking care not to transfer any lower layers.
- 23.6.10 Evaporate to dryness at approximately 50° C under nitrogen.
- 23.6.11 Reconstitute samples in 100 µL acetonitrile. Vortex briefly. Transfer to GC microvials.

### 23.7 Calculation

- 23.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.

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<p><b>23.8 Quality Control and Reporting</b></p> <p>23.8.1 See Toxicology Quality Guidelines</p> <p><b>23.9 REFERENCES</b></p> <p>23.9.1 J Pearson and R Steiner, in-house development.</p>	